Effects of ectomycorrhizae and solution pH on [^{15}N]nitrate uptake by coniferous seedlings

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The interacting effects of mycorrhizae and pH on nitrate uptake by three native Northwest coniferous species were examined. Seedlings of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), Sitka spruce (*Picea sitchensis* (Bong.) Carr.), and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) were grown for several months, and then half of the seedlings were inoculated with the ectomycorrhizal fungus *Hebeloma crustuliniforme* (Bull. ex St. Amans) Quél. In a series of experiments, pH was continuously maintained at set levels and fluxes of nitrate, potassium, calcium, and hydroxide ions were measured. While both coniferous species and mycorrhizae did affect nitrate uptake rates, the major effect on nitrate uptake rates was due to solution pH. Nitrate uptake rates generally increased with increasing pH from pH 2.5 to 7.5, as did calcium flux rates, which changed from efflux at low pH to uptake at higher pH levels. Potassium was released from the roots at all pH levels. Hydroxide ion release rates decreased with increasing pH, resulting in hydrogen ion release above pH 5.5. Mycorrhizal roots often released fewer hydroxide ions per nitrate ion taken up than did nonmycorrhizal roots, leading to the suggestion that mycorrhizae may act as rhizosphere buffers. Among the three coniferous species, Douglas-fir roots released more hydroxide ions per nitrate taken up than did western hemlock; Sitka spruce values were intermediate. These apparent species effects may be related to the less acidic mineral soil environment where Douglas-fir roots are often found, and to the more acidic forest floor environment where western hemlock roots primarily grow.

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Les auteurs ont examiné les effets interactifs des mycorhizes et du pH sur l'absorption du nitrate par trois espèces conifériennes du nord-ouest américain. Des plantules de sapin de Douglas (*Pseudotsuga menziesii* (Mirb.) Franco), d'épinette de Sitka (*Picea sitchensis* (Bong.) Carr.) et de pruche de l'ouest (*Tsuga heterophylla* (Raf.) Sarg.) ont été cultivées pendant plusieurs mois, après quoi la moitié des plantules ont été inoculées avec le champignon ectomycorhizien *Hebeloma crustuliniforme* (Bull. ex St. Amans) Quél. Dans une série d'expériences, le pH a été maintenu à un ensemble de valeurs et les mouvements des ions nitrate, potassium, calcium et hydroxyde ont été mesurés. Bien que les espèces conifériennes aussi bien que la présence des mycorhizes ont affecté les taux d'absorption des nitrates, le principal effet était dû au pH de la solution. Les taux d'absorption du nitrate augmentaient généralement avec une augmentation de la valeur du pH de 2,5 à 7,5, comme c'était le cas avec les mouvements du calcium qui a passé d'un efflux au faible pH à une absorption aux pH élevés. Les racines ont relâché du potassium à tous les pH. Les taux de relâchement d'ions hydroxyde ont diminué avec une augmentation du pH, entraînant un sortie d'ion hydrogène au-dessus du pH 5,5. Souvent les racines mycorhizées ont relâché moins d'ions hydroxyde par ion nitrate absorbé que ne l'ont fait les racines nonmycorhizées, ce qui suggère que les mycorhizes pourraient agir comme un tampon rhizosphérique. Parmi les trois espèces de conifères, les racines de sapin de Douglas ont relâché plus d'ions hydroxyde par ion nitrate absorbé que ne l'a fait la pruche de l'ouest; pour l'épinette de Sitka les valeurs étaient intermédiaires. Cette diversité apparente entre les espèces pourrait être reliée aux conditions moins acides du sol là où se retrouvent les racines du sapin de Douglas et au parterre forestier plus acide où se développent surtout les racines de la pruche de l'ouest.

[Traduit par le journal]

Introduction

Data on NO_3^- uptake by agricultural plant species abound in the literature, whereas little data exist for NO_3^- uptake by coniferous species. This is due in part to the NO_3^- preference shown by agricultural plants (Huffaker and Rains 1978). Generally, the slightly acidic to basic soils in which agricultural plants grow are higher in NO_3^- than in NH_4^+, and these plants appear to grow best when nitrogen is supplied as NO_3^-. In coniferous forest soils, NH_4^+ is the predominant nitrogen form (Vogt and Edmonds 1982), and coniferous species generally grow best when NH_4^+ is the nitrogen source (van den Driessche 1971). We have previously examined NH_4^+ uptake by coniferous species (Littke 1982; Bledsoe and Zasoski 1983; Rygiewicz et al. 1984). Although NO_3^- levels in forest soils are usually low, NO_3^- is found in acidic forest soils (Vogt and Edmonds 1982), and does provide an additional nitrogen source. In this paper we report on NO_3^- uptake by three native Northwest coniferous species.

Mycorrhizae have been shown to increase ion uptake rates, particularly for phosphorus (Bowen 1973). Studies have demonstrated a mycorrhizal enhancement of NH_4^+ uptake for several coniferous species (France 1980; Littke 1982; Rygiewicz 1983; Bledsoe and Zasoski 1983), but there is little data for NO_3^-. Therefore the ability of mycorrhizae to alter NO_3^- uptake was considered in this study. Solution pH effects on NO_3^- acquisition should be considered since NO_3^- uptake will usually increase solution pH. These solution pH changes external to the root can affect subsequent nutrient uptake via competition between OH^- or HCO_3^- and NO_3^-. Of the literature examining pH effects on nitrogen uptake, few studies were done where the external pH changes were controlled during the uptake period.

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TABLE 1. Composition of uptake and preuptake solutions with added micronutrients (as indicated in the Materials and methods, Pretreatment section)

Compound	Concentration (m*M*)	Ion	Ion concentration ($\mu Eq \cdot L^{-1}$)
$K^{15}[NO_3]$	0.7	K^+ and NO_3^-	700
$CaCl_2$	0.4	Ca^{2+}	800
Na_2HPO_4	0.03	$H_2PO_4^-$*	30
$MgSO_4 \cdot 7H_2O$	0.01	Mg^{2+}	20

*This is the predominant ionic species in the pH range 2.5–6.5, while at pH 7.5, both $H_2PO_4^-$ and HPO_4^{2-} are present.

In this study, pH was continuously maintained at a constant value during NO_3^- uptake by mycorrhizal and nonmycorrhizal roots of intact coniferous seedlings. Because calcium and potassium fluxes may be affected by NO_3^- uptake and they can be important in maintaining charge balance, their uptake was also measured.

Materials and methods

Plant material

Seedlings of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) were grown from seed in a greenhouse as described previously (Rygiewicz et al. 1984). Seedlings were fertilized weekly with a complete nutrient solution (Rygiewicz 1983). Nitrogen was supplied as NH_4NO_3 at 7 m*M* initially; after inoculation the N rate was reduced to 1.4 m*M*. Half of the seedlings were inoculated with the ectomycorrhizal fungus *Hebeloma crustuliniforme* (Bull. ex St. Amans) Quél (Littke et al. 1980). Mycorrhizal hemlock seedlings were stolen from the greenhouse and were not available for experimentation.

Experimental design

The experiment was designed to test the influence of mycorrhizae and pH on NO_3^- uptake by seedlings of three coniferous species. Each species, with and without mycorrhizae, was tested at six pH values: 2.5, 3.5, 4.5, 5.5, 6.5, and 7.5. Each treatment was replicated four times on pairs of seedlings and all factors were fully randomized. A total of 288 seedlings was produced.

Pretreatment and apoplastic adsorption

Washed roots of intact seedlings were pretreated for 48 h in dilute nutrient solution which contained micronutrients (1:100 dilution of Johnson's solution, Epstein 1972) and macronutrients (0.04 m*M* $NaNO_3$, 0.01 m*M* K_2HPO_4, 0.4 m*M* $CaCl_2$, and 0.01 m*M* $MgSO_4 \cdot 7H_2O$). Pretreatment solution pH was initially the same as the subsequent uptake solution pH but was allowed to vary during pretreatment. Prior to the uptake period, seedling roots were placed in a preuptake solution for 15 min to allow CW + FS (cell wall + free space) exchange to occur. Details of pretreatment and adsorption have been described previously (Rygiewicz et al. 1984).

Uptake and apoplastic desorption

Roots of intact seedlings were placed in the uptake solution at 18°C with aeration for 4 h (see Table 1 for solution composition). A concentration of 0.7 m*M* nitrate (99% enrichment of stable isotope ^{15}N) was chosen to maximize nitrogen uptake. For Douglas-fir seedlings in solution cultures, optimum NO_3^- concentration was approximately 0.2–1.0 m*M* NO_3^- (Littke 1982). This concentration also approximates NO_3^- levels (0.01–0.90 m*M* NO_3^-) found in Pacific Northwest forest soils (Vogt and Edmonds 1982). Solution pH was maintained by addition of 0.01 *N* HCl with an automatic titrating pH stat. After the uptake period, roots were desorbed for 15 min at 2°C in nitrogen-free uptake solution (Rygiewicz et al. 1984).

Analysis

Seedlings were separated into roots and shoots, oven dried, and weighed. Roots were ground in liquid nitrogen and both total nitrogen (microKjeldahl method) and ^{15}N (mass spectrometric method) were determined as described previously (Rygiewicz et al. 1984). Net flux (uptake or release) of Ca^{2+} and K^+ was calculated as the difference between initial and final nutrient concentrations (determined by atomic absorption) of the uptake solution. Release of OH^- from seedling roots was calculated using the amount and concentration of HCl dispensed by the autoburette during the uptake period. This method does not distinguish between the release of base (OH^- and HCO_3^-) or H^+ uptake; however, base release was assumed to be the most likely alternative. In this paper the net result of H^+ added by titration is referred to as OH^- release.

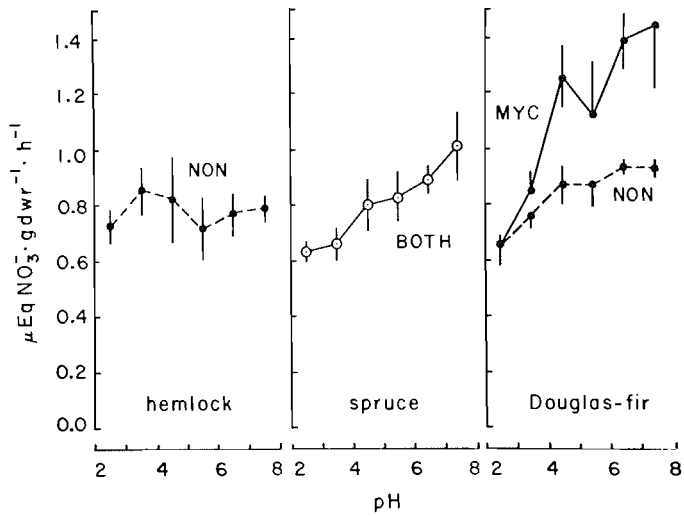

FIG. 1. Nitrate uptake by nonmycorrhizal (NON) and mycorrhizal (MYC) coniferous seedlings as a function of pH. Data points are means of four replications with standard errors indicated. Only nonmycorrhizal hemlock data were available. Seedling types (NON and MYC) for spruce were not significantly different ($p > 0.05$), and data were combined (BOTH, eight replicates per datum point; the symbol ○ indicates pooled data).

Statistical analyses

Analyses of covariance were conducted to determine if effects of three major variables on nutrient fluxes were significant. The three major variables were (*i*) presence of mycorrhizae, (*ii*) solution pH, and (*iii*) coniferous species, with root and total seedling dry weights and R:S (root:shoot) included as covariates (SPSS, Statistical Package for the Social Sciences, Nie et al. 1975). Duncan's new multiple range test (Steel and Torrie 1960) was used to identify significant differences among individual treatment means. Graphical presentations of the results were facilitated by separating the coniferous species and analyzing each species separately for mycorrhizal effects. In the Results section, significant differences are indicated as $p \leq 0.05$, significant; $p \leq 0.01$, highly significant; and $p \leq 0.001$, very highly significant.

Results

The analyses of covariance of nutrient fluxes indicated significant three-way interactions between the mycorrhizal, coniferous species, and pH treatments. The small variation in seedling biomass or R:S (covariates) within a coniferous species did not consistently affect nutrient fluxes as indicated by analyses of covariance. In Figs. 1, 2, 4, 5, and 6, efflux of ions is presented as a negative value and influx of ions is presented as a positive value.

Root tips of mycorrhizal seedlings were approximately 40–50% mycorrhizal compared with less than 1% infection for uninoculated controls. Between mycorrhizal and nonmycor-

TABLE 2. Seedling characteristics for mycorrhizal (+) and uninoculated control (−) plants. Values are means of 24 replicates ± SE, except potassium and calcium values which are means of 6 replicates ± SE

Coniferous species	Mycorrhizal status	Seedling dry weight	Root:shoot	Nutrient concentration in roots		
				%N	%K	%Ca
Douglas-fir	+	1.7±0.08	0.73±0.04	0.64±0.02	0.42±0.02	0.17±0.04
	−	1.6±0.08	0.70±0.04	0.58±0.02	0.41±0.03	0.16±0.05
Hemlock	+	NA*	NA	NA	NA	NA
	−	2.5±0.05	0.28±0.01	0.97±0.02	0.87±0.08	0.31±0.08
Spruce	+	2.1±0.09	0.70±0.03	0.69±0.02	0.76±0.07	0.31±0.08
	−	1.8±0.11	0.67±0.02	0.70±0.02	0.86±0.07	0.30±0.07

*NA, mycorrhizal hemlock seedlings were not available for experimentation.

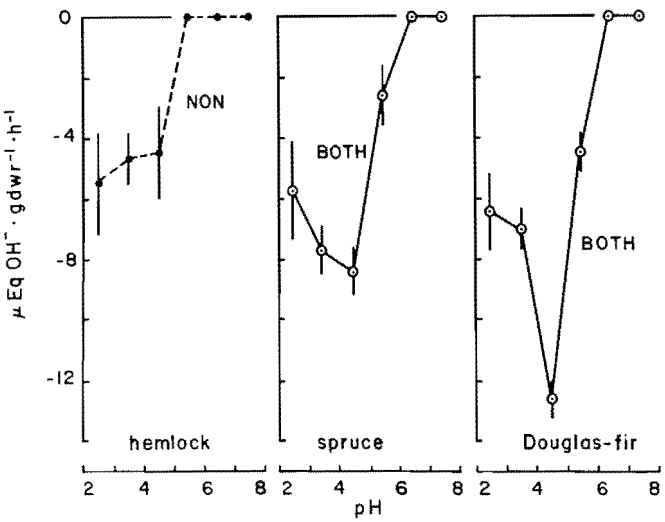

FIG. 2. Flux of OH^- during NO_3^- uptake as a function of pH. All conventions as in Fig. 1.

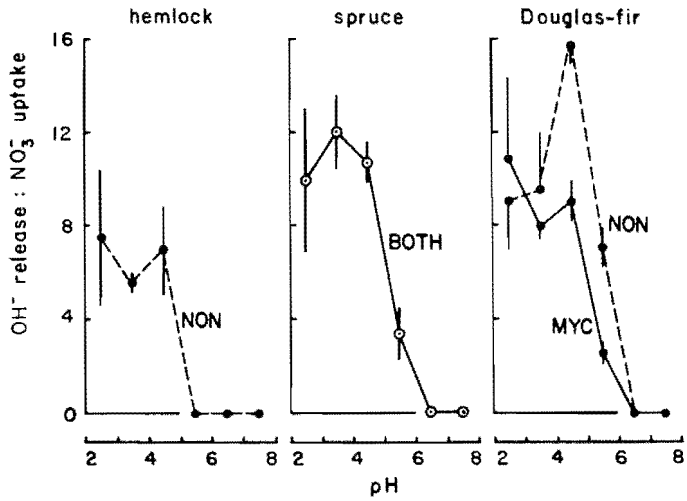

FIG. 3. The ratio of OH^- flux to NO_3^- flux as a function of pH. All conventions as in Fig. 1.

rhizal seedlings, seedling total dry weights, R:S ratio, and root percent nitrogen, potassium, and calcium contents were similar (Table 2). As expected, seedling characteristics for Douglas-fir, hemlock, and spruce were different. These differences were considered in the statistical treatment of the data.

Nitrate uptake

Nitrate uptake generally increased with increasing pH (Fig. 1). This pH effect was dependent upon the presence of mycorrhizae as well as coniferous species. Nitrate uptake rates increased with increasing pH for all spruce and Douglas-fir seedlings, but pH had no effect on NO_3^- uptake rates by nonmycorrhizal hemlock. Mean NO_3^- uptake rates of all nonmycorrhizal seedlings were similar among the three coniferous species. There was a very highly significant mycorrhizal effect on NO_3^- uptake for Douglas-fir but not for spruce (Fig. 1). Therefore, nonmycorrhizal and mycorrhizal spruce data were combined. At higher pH levels, the NO_3^- uptake rates for mycorrhizal Douglas-fir were greater (highly significant) than respective nonmycorrhizal rates, but below pH 3.5 there was no difference.

Ion fluxes during NO_3^- uptake

Hydroxide

Because plant cells must maintain charge balance as NO_3^- is acquired, other ions must be transferred between the roots and the external solution. During NO_3^- uptake, OH^- efflux increased as pH decreased (Fig. 2). Below pH 4.5, OH^- efflux occurred for all seedlings. At pH 5.5 hemlock did not release OH^-, and above pH 5.5 there was no OH^- release from any seedling roots. Mycorrhizae did not significantly affect OH^- release rates for either Douglas-fir or spruce, so nonmycorrhizal and mycorrhizal data were combined for graphical presentation. Douglas-fir seedlings released OH^- at significantly faster rates than spruce (mean rate; 5.1 vs. 4.1 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$) (gdwr, grams of dry weight root). Data for all three nonmycorrhizal species were compared and the mean release rates were 5.5, 4.5, and 2.5 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$, respectively, for Douglas-fir, spruce, and hemlock. In general, OH^- efflux during NO_3^- uptake was characterized by a maximum release rate at pH 4.5. Rates decreased to intermediate levels at more acidic pH and decreased to zero at less acidic pH.

When OH^- efflux data were normalized for corresponding NO_3^- uptake rates, the $OH^-:NO_3^-$ ratios (OH^- ions released per NO_3^- ions taken up) were lowest for nonmycorrhizal hemlock followed by nonmycorrhizal spruce and Douglas-fir (3.4, 6.5, and 6.9, respectively, mean for each species across all pH levels combined). Only mycorrhizal Douglas-fir seedlings were significantly different from respective nonmycorrhizal seedlings (Fig. 3). Significant differences for $OH^-:NO_3^-$ ratios between mycorrhizal and nonmycorrhizal Douglas-fir seedlings were found at pH 4.5 and 5.5; above or below those pH's there was no difference between seedling types.

TABLE 3. Effects of mycorrhizae on ion fluxes during NO_3^- uptake for the three coniferous species. Individual ion flux values (microequivalents per grams of dry weight root per hour) are positive if the ion was taken up by the root and negative if it was released to the solution

Coniferous species	Nitrate*			Hydroxide*			Calcium*			Potassium*			Σ, measured flux*†		
	NON	MYC	$\bar{X}$	NON	MYC	$\bar{X}$	NON	MYC	$\bar{X}$	NON	MYC	$\bar{X}$	NON	MYC	$\bar{X}$
Spruce	0.84	0.76	0.80	−4.5	−3.7	−4.1	−0.09	−0.75	−0.42	−8.1	−4.6	−6.4	−4.5	−2.4	−3.5
Douglas-fir	0.84	1.1	0.98	−5.5	−4.7	−5.1	−5.1	−2.4	−3.7	−6.7	−3.1	−4.9	−7.1	−1.9	−4.5
Hemlock	0.77	—	0.77	−2.5	—	−2.5	6.2	—	6.2	−11.0	—	11.0	−3.1	—	−3.1

*Fluxes of NO_3^-, Ca^{2+}, K^+ and the Σ between nonmycorrhizal (NON) and mycorrhizal (MYC) seedling types for all species were significantly different ($p \leq 0.05$), while OH^- fluxes were not.
†To calculate the measured ion flux, which represents the charge within the root, the following equation was used: total flux = Ca^{2+} flux + K^+ flux − NO_3^- flux − OH^- flux.

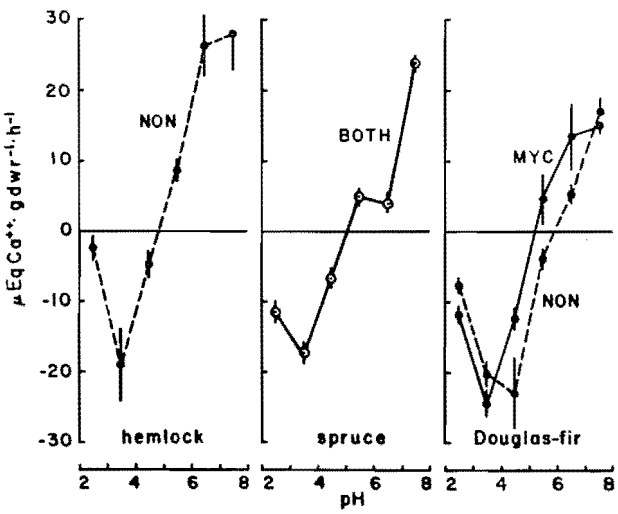

FIG. 4. Flux of Ca^{2+} during NO_3^- uptake as a function of pH. All conventions as in Fig. 1.

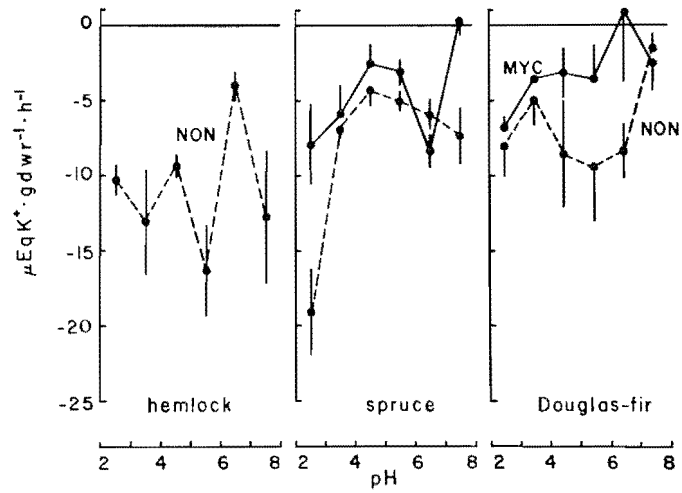

FIG. 5. Flux of K^+ during NO_3^- uptake as a function of pH. All conventions as in Fig. 1.

Calcium ion

Calcium flux changed from efflux at lower pH's to uptake at higher pH's (Fig. 4). All seedlings released Ca^{2+} most rapidly at pH 3.5, except nonmycorrhizal Douglas-fir seedlings which had a maximal release rate at pH 4.5. At a more acidic pH, release rates dropped to intermediate levels. At pH levels 5.5 or above, efflux ceased and net Ca^{2+} uptake occurred. Mycorrhizae had a significant effect only for Douglas-fir, which resulted in a shift of the pH level at which Ca^{2+} efflux ceased. Although the patterns of Ca^{2+} flux were similar, significant species effects were found for both seedling types. When the data for all pH levels were combined (Table 3), Douglas-fir released Ca^{2+}, while hemlock took up Ca^{2+}, and spruce Ca^{2+} fluxes were essentially zero.

Potassium ion

Flux of K^+ was highly variable and relatively independent of pH (Fig. 5). Efflux of K^+ occurred for all seedlings at all pH levels, except at pH 6.5 (mycorrhizal Douglas-fir) and pH 7.5 (mycorrhizal spruce). Above pH 2.5, pH had little effect on K^+ efflux by nonmycorrhizal hemlock, spruce, or Douglas-fir. When nonmycorrhizal seedlings were compared, hemlock released K^+ at significantly faster rates than Douglas-fir; spruce was intermediate and not significantly different from either (mean rates were 11.0, 8.1, and 6.7 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$ for hemlock, spruce, and Douglas-fir, respectively). Mycorrhizal seedlings generally had lower K^+ efflux rates than nonmycorrhizal seedlings across the pH range.

Ion fluxes

The sum of measured ion fluxes, (Ca^{2+} + K^+ − (NO_3^- + OH^-) reflects net charge within the root cell. The effects of mycorrhizae on these sums, averaged across all pH experiments, are shown in Table 3. These sums were not zero, suggesting that movement of other ions occurred to maintain charge balance. Solution pH also affected total ion flux in a very highly significant manner for all three species (Fig. 6). Below pH 5.5−6.5, large release rates for Ca^{2+} and K^+ were recorded, and measured ion fluxes were negative. As pH increased, total measured flux (NO_3^-, OH^-, Ca^{2+}, and K^+) became less negative until above 5.5−6.5 net cation uptake predominated. Above pH 5.5−6.5, OH^- efflux no longer occurred, since the pH of the external solution dropped rather than increased. It was necessary to manually maintain a constant pH by additions of NaOH (the pH stat controlled only HCl additions). Mycorrhizae significantly affected total measured ion flux, but only for Douglas-fir and only at solution pH 5.5−6.5. Above or below this pH range, there was no significant mycorrhizal effect. When only nonmycorrhizal or only mycorrhizal seedlings were analyzed, there were no significant differences among coniferous species. From these data it appears that, for both seedling types, large fluxes of unmeasured ions occurred.

Discussion

Solution pH effects on nitrate uptake

Limited data are available to compare coniferous seedling NO_3^- uptake rates in solution culture. Littke (1982) measured a mean uptake rate of 1.0 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$ (solution concentration = 0.7 m*M* NO_3^-) for nonmycorrhizal and mycorrhizal Douglas-fir seedlings at pH 5.8). Martin et al. (1981) reported

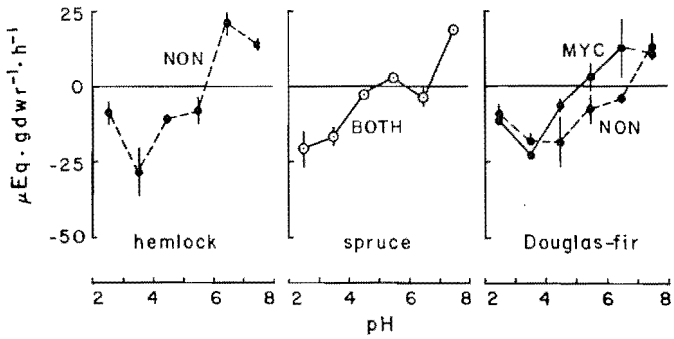

FIG. 6. Measured ion flux ($Ca^{2+} + K^+ - (NO_3^- + OH^-)$) as a function of pH. All conventions as in Fig. 1.

NO_3^- uptake rates of 2.4 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$ for 5-week-old *Pinus nigra* seedlings in 5 m*M* NO_3^-. In our study, an average value for all species was approximately 1 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$ at 0.7 m*M* NO_3^-. In contrast with these somewhat low rates for coniferous seedlings, wheat plants had rates of about 40–60 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$ (Jackson et al. 1976).

Nitrate uptake generally increased with increasing solution pH (Fig. 1). This is surprising since most reports indicate that NO_3^- uptake rates for most species decrease with increasing solution pH (Moore 1974; Rufty et al. 1982). Plant roots also release OH^- during NO_3^- uptake (Raven and Smith 1976) and this was observed in our study as well. There are at least two possible reasons for the different response of NO_3^- uptake to pH between these conifers and other primarily agricultural species.

First, the pH optimum for growth of the fungus *H. crustuliniforme* has been reported as pH 5 (buffered solution, Littke et al. 1984) or as pH 8 (unbuffered solution, Hung and Trappe 1983). The pH optimum for growth of conifers is generally lower, pH 5.5 or below (van den Driessche 1978). Thus the increased NO_3^- uptake rates for mycorrhizal Douglas-fir seedlings at higher pH may be due to pH effects on NO_3^- uptake by the fungus. Secondly, increased Ca^{2+} uptake with increasing pH (Fig. 4) may have increased uptake of the counter ion NO_3^- to maintain charge balance. Because NO_3^- uptake was only approximately 7% of the measured ion fluxes, NO_3^- uptake may have been affected primarily by cation uptake and less by solution pH.

When comparing NH_4^+ nutrition with NO_3^- nutrition, more cations are absorbed during NO_3^- uptake for conifers (Cole 1981) and for agricultural species (Dejaegere et al. 1981). Conifers are NH_4^+-based plants and, relative to agricultural species, they absorb more cations per NH_4^+ taken up (Rygiewicz 1983). We assume that conifers take up more cations during NO_3^- uptake than agricultural species, and that NO_3^- uptake is primarily coupled to cation uptake, not to pH. This lesser dependency of NO_3^- on pH may explain why NO_3^- uptake does not follow the agricultural plant pattern of decreasing NO_3^- uptake with increasing pH.

Mycorrhizal effects on NO_3^- uptake

Only mycorrhizal Douglas-fir seedlings had significantly higher rates compared with respective nonmycorrhizal plants (1.3 times faster). Considerable work has been done on mycorrhizal enhancement of nitrogen nutrition but little of it pertains to coniferous species (see review by Bowen and Smith 1981). Littke (1982) reported that mycorrhizal Douglas-fir seedlings absorbed more NO_3^- than did nonmycorrhizal seedlings. He concluded that mycorrhizae can increase both the affinity and capacity for NO_3^- uptake; however, NO_3^- uptake rates were much less than the NH_4^+ uptake rates for the seedling roots. Littke et al. (1984) also reported that the NO_3^- uptake rate by the fungus alone was much less than the NH_4^+ rate. The lack of an enhancement of NO_3^- rates by mycorrhizal spruce seedlings may indicate that mycorrhizae may play a less prominent role in acquisition of nutrients, such as NO_3^-, which are more mobile in soil systems.

Ratio of OH^-:NO_3^-

Release of OH^- during NO_3^- uptake observed in this study is in agreement with widely observed increases in solution and rhizosphere pH during NO_3^- uptake (Smiley 1974; Raven and Smith 1976; Bledsoe and Zasoski 1983; and many others). The amount of OH^- released per NO_3^- taken up (OH^-:NO_3^-) may differ depending on the plant species and the presence of mycorrhizae. For some higher plants, OH^-:NO_3^- ratios range from 0.05 to 0.9 depending upon the operation of the biochemical pH stat (Raven and Smith 1976). Dejaegere et al. (1981) determined that the OH^-:NO_3^- ratio for several agricultural species was approximately 0.5. The grand mean OH^-:NO_3^- ratios for the coniferous species of this study was 5.5 and was pH dependent (from 3.3 at pH 5.5 to 11 at pH 4.5, all seedling data combined, Fig. 3). Our data suggest that coniferous seedlings probably maintain ionic balance in a manner different from agricultural plant species.

Because mycorrhizal Douglas-fir seedlings took up nitrogen at faster rates, we were curious whether this could lead to greater changes in external pH for this species. When OH^- release rates were standardized to NO_3^- uptake, significantly fewer OH^- were released per NO_3^- taken up for mycorrhizal Douglas-fir. Smaller OH^-:NO_3^- ratios for mycorrhizal Douglas-fir seedlings may have been due to a different dependency on the biochemical pH stat (Raven and Smith 1976) or to higher cation uptake rates by mycorrhizal seedlings.

Regardless of the way mycorrhizal seedlings achieve different OH^-:NO_3^- stoichiometric relations, mycorrhizae appear to act as rhizosphere buffers. For an equal amount of NO_3^- absorption, external pH was altered less. This phenomenon of smaller pH change with mycorrhizae was also observed during NH_4^+ uptake (Rygiewicz et al. 1984). This buffering could be important in situations where pH changes would lead to altered solubilities of other ions in the rhizosphere. For example, if anion (A) exceeds cation (C) uptake ($C - A < 0$), less OH^- release by mycorrhizae could affect phosphate, iron, and aluminum solubilities (Moore 1974).

Charge balance

Ionic balance of plant roots requires maintenance of charge balance resulting from differential ion fluxes (Kirkby 1969). For the species in this study, total measured ion flux for all treatments was negative at low pH and became positive as pH increased (Fig. 6). We measured fluxes of NO_3^-, OH^-, K^+, and Ca^{2+}; obviously there was a need for fluxes of other ions, which we did not measure, to maintain charge balance.

At low pH, measured ion flux was negative owing to cation release (Ca^{2+} and K^+). The seedling roots could maintain charge balance at low pH by either anion release or cation uptake. All cations of appreciable concentration in solution were measured so that uptake of other cations was not a possible mechanism. We did not measure these anions, i.e., HCO_3^-, $H_2PO_4^-$, SO_4^{2-}, Cl^-, or organic anions. A release of these anions at low pH would result in charge balance. In related work, we have measured release of both HCO_3^- (Rygiewicz 1983) and

SO_4^{2-} (Rygiewicz et al. 1984) during ammonium uptake by Douglas-fir roots. These related studies indicate that anion release from coniferous species roots is a possible mechanism to maintain charge balance.

At high pH, measured ion flux was positive, due primarily to Ca^{2+} uptake. Maintenance of charge balance for these coniferous species at the higher pH values may have been achieved by release of H^+ or increased anion uptake. We were unable to measure H^+ release during NO_3^- uptake since the pH stats were titrating HCl. Instead, solution pH was controlled by manual additions of NaOH at these pH levels. We suspect that charge balance was maintained at higher pH by the release of H^+ rather than by increased anion uptake.

Potassium was released for all treatments, whereas Ca^{2+} was released at low pH and absorbed above pH 4.5–6. Potassium fluxes were more variable across pH and usually larger than Ca^{2+} fluxes. Slower Ca^{2+} flux compared with K^+ was as predicted for mono- and di-valent ions (Hiatt and Leggett 1974). We recognize that K^+ and Ca^{2+} release must be a short-term phenomenon and that these plants are K^+ and Ca^{2+} accumulators. Although K^+ efflux may have resulted from membrane damage at low pH, the occurrence of NO_3^- uptake at these pH argues against this possibility. No doubt the rapid K^+ release for spruce at pH 2.5 was anomalous and deserves further attention. Calcium was released from pH 2.5–4.5. It is difficult to imagine coniferous root membranes adversely affected over this pH range, since the pH of forest soils can equal these values. Part of the Ca^{2+} and K^+ release at low pH may have been due to exchange in the CW + FS with H^+.

Species comparisons

Mean NO_3^- uptake rates among nonmycorrhizal seedlings were similar and apparently not related to the respective growth rates for each species. Douglas-fir and Sitka spruce seedlings have been shown to grow more rapidly than hemlock seedlings (van den Driessche 1971). Although NO_3^- uptake rate and growth rate appear to be uncoupled, this is not true for NH_4^+ uptake. Rygiewicz et al. (1984) reported that NH_4^+ uptake rates for the faster-growing Douglas-fir and spruce were more rapid than rates for the slower-growing hemlock. F. S. Chapin (personal communication) has suggested that there is a positive correlation between growth rates and uptake of immobile ions (such as PO_4^- and NH_4^+), while there is a negative correlation between growth rate and uptake of more mobile ions (such as K^+ or NO_3^-). Our data support his suggestion for the immobile ions, but not for the mobile ions.

The release of OH^- per NO_3^- absorbed ($OH^-:NO_3^-$ ratio) should affect the pH of the soil and rhizosphere in which roots reside. Roots which have higher $OH^-:NO_3^-$ ratios should tend to raise the soil pH more than roots with lower $OH^-:NO_3^-$ ratios. In our study hemlock seedlings had the lowest ratio, while ratios of Douglas-fir and spruce were greater. Hemlock has been shown to grow in more acidic soils than Douglas-fir and to tolerate these acidic soils better than Douglas-fir (Ryan 1983; Heilman and Ekuan 1973). Our data on NH_4^+ uptake (Rygiewicz et al. 1984) indicated that hemlock may further acidify soils, since hemlock had higher $H^+:NH_4^+$ ratios than either Douglas-fir or Sitka spruce. In contrast, Douglas-fir, which normally roots in mineral soils which have higher pH than the forest floor, had the lowest $H^+:NH_4^+$ ratio and the highest $OH^-:NH_4^+$ ratio (Rygiewicz et al. 1984; this paper, Fig. 3). Al these data imply a relationship between edaphic conditions and nitrogen accumulation in these conifers.

Summary

Mycorrhizae significantly increased NO_3^- uptake rates compared with respective controls only for Douglas-fir. Mycorrhizae had a much greater influence on NH_4^+ uptake rates (Rygiewicz et al. 1984) than on NO_3^- rates, since all three species when mycorrhizal had increased NH_4^+ uptake rates. In both cases, uptake increased with increasing pH. Mycorrhizae generally lowered the $OH^-:NO_3^-$ ratios for coniferous seedlings, suggesting that mycorrhizae may act as rhizosphere buffers. Equivalent amounts of NO_3^- can be taken up by mycorrizal roots with less change in rhizosphere pH. Similar results for NH_4^+ uptake have been reported (Rygiewicz et al. 1984). This research supports the concept that mycorrhizae benefit plants by increasing ion uptake, but the role of mycorrhizae for acquisition of the more mobile nutrients (such as NO_3^+) may be less important than for less mobile ions (such as NH_4^+).

Nitrate uptake rates by western hemlock, Sitka spruce, and Douglas-fir seedlings were not greatly affected by solution pH. Solution pH had greater effects on NH_4^+ uptake than on NO_3^- uptake. Even though NO_3^- uptake significantly increased with increasing solution pH, the effect was modest and all rates were relatively low (only about 7% of the measured ion flux). The increase in NO_3^- rates with increasing pH may have been a counter ion effect with NO_3^- rates dependent primarily on Ca^{2+} uptake rates. Nitrate uptake rates by these coniferous seedlings were much slower than rates reported for agricultural species, supporting the contention that ammonium is the primary nitrogen source for these conifers.

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